

This Page Is Inserted by IFW Operations
and is not a part of the Official Record

BEST AVAILABLE IMAGES

Defective images within this document are accurate representations of the original documents submitted by the applicant.

Defects in the images may include (but are not limited to):

- BLACK BORDERS
- TEXT CUT OFF AT TOP, BOTTOM OR SIDES
- FADED TEXT
- ILLEGIBLE TEXT
- SKEWED/SLANTED IMAGES
- COLORED PHOTOS
- BLACK OR VERY BLACK AND WHITE DARK PHOTOS
- GRAY SCALE DOCUMENTS

IMAGES ARE BEST AVAILABLE COPY.

**As rescanning documents *will not* correct images,
please do not report the images to the
Image Problem Mailbox.**

At page 1, top, delete " EXTENDED RHODAMINE COMPOUNDS USEFUL AS
FLUORESCENT LABELS", and insert therefor NUCLEIC ACIDS LABELLED
WITH EXTENDED RHODAMINE DYES --

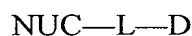
At page 1, after the title, please insert: This application is a division of Application No.
09/325,243, filed June 3, 1999, which is incorporated herein by reference.--

In the claims:

Please add new claims 46-75.

Please cancel claims 1-45 without prejudice.

-- 46. A labelled nucleic acid compound having the formula:

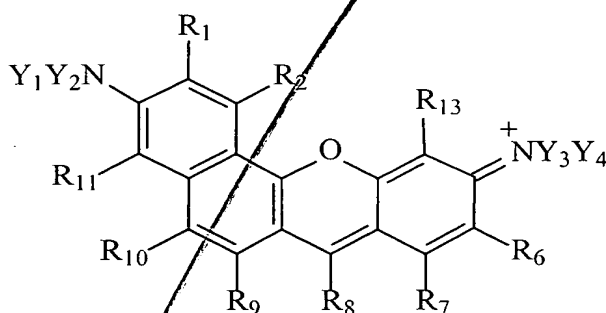
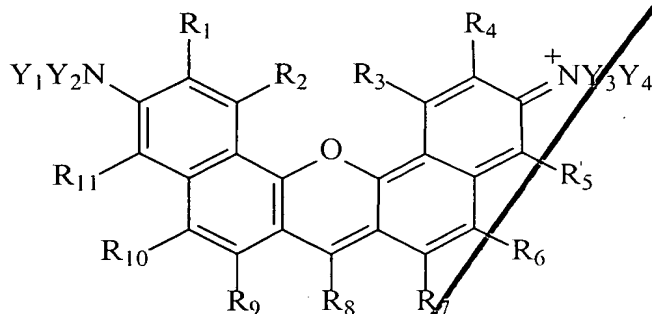


wherein

NUC is a nucleic acid compound selected from a nucleoside, a nucleotide, a polynucleotide and analogs thereof;

L is a linkage; wherein if NUC comprises a purine base, the linkage is attached to the 8-position of the purine, if NUC comprises a 7-deazapurine base, the linkage is attached to the 7-position of the 7-deazapurine, and if NUC comprises a pyrimidine base, the linkage is attached to the 5-position of the pyrimidine; and

D is an extended rhodamine dye comprising the structures:



wherein

R_1 , R_2 , R_3 , R_4 , R_5 , R_6 , R_7 , R_9 , R_{10} , R_{11} , and R_{13} when taken alone are selected from -H, alkyl, alkyl independently substituted with one or more Z_1 , heteroalkyl, heteroalkyl independently substituted with one or more Z_1 , aryl, aryl independently substituted with one or more Z_1 , heteroaryl, heteroaryl independently substituted with one or more Z_1 , arylalkyl, arylalkyl independently substituted with one or more Z_1 , heteroarylalkyl, heteroarylalkyl independently substituted with one or more Z_1 , halogen, $-\text{OS}(\text{O})_2\text{OR}$, $-\text{S}(\text{O})_2\text{OR}$, $-\text{S}(\text{O})_2\text{R}$, $-\text{S}(\text{O})_2\text{NR}$, $-\text{S}(\text{O})\text{R}$, $-\text{OP}(\text{O})\text{O}_2\text{RR}$, $-\text{P}(\text{O})\text{O}_2\text{RR}$, $-\text{C}(\text{O})\text{OR}$, $-\text{NR}_2$, $-\text{NR}_3$, $-\text{NC}(\text{O})\text{R}$, $-\text{C}(\text{O})\text{R}$, $-\text{C}(\text{O})\text{NR}_2$, $-\text{CN}$, and $-\text{OR}$, wherein R is independently selected from -H, alkyl, heteroalkyl, aryl, heteroaryl, arylalkyl, heteroarylalkyl and linking group; or

R_1 taken together with R_2 , Y_1 , or Y_2 ; or

R_4 taken together with R_3 , Y_3 , or Y_4 ; or

R_5 taken together with R_6 , Y_3 , or Y_4 ; or

R_6 taken together with R_7 , Y_3 , or Y_4 ; or

R_{10} taken together with R_9 or R_{11} ; or

R_{11} taken together with Y_1 , or Y_2 ; or

R₁₃ taken together with Y₃ or Y₄

are selected from alkyleno, alkyleno independently substituted with one or more Z₁, heteroalkyleno, heteroalkyleno independently substituted with one or more Z₁, aryleno, aryleno independently substituted with one or more Z₁, heteroaryleno, and heteroaryleno independently substituted with one or more Z₁;

R₈ is selected from -H, alkyl, alkyl independently substituted with one or more Z₁, heteroalkyl, heteroalkyl independently substituted with one or more Z₁, aryl, aryl independently substituted with one or more Z₁, heteroaryl, heteroaryl independently substituted with one or more Z₁, arylalkyl, arylalkyl independently substituted with one or more Z₁, heteroarylalkyl, and heteroarylalkyl independently substituted with one or more Z₁;

Y₁, Y₂, Y₃, Y₄ when taken alone are selected from -H, alkyl, alkyl independently substituted with one or more Z₁, heteroalkyl, heteroalkyl independently substituted with one or more Z₁, aryl, aryl independently substituted with one or more Z₁, heteroaryl, heteroaryl independently substituted with one or more Z₁, arylalkyl, arylalkyl independently substituted with one or more Z₁, heteroarylalkyl, and heteroarylalkyl independently substituted with one or more Z₁; or

Y₁ taken together with R₁, R₁₁ or Y₂; or

Y₂ taken together with R₁, R₁₁ or Y₁; or

Y₃ taken together with R₄, R₅, R₆, R₁₃ or Y₄; or

Y₄ taken together with R₄, R₅, R₆, R₁₃ or Y₃

are selected from alkyleno, alkyleno independently substituted with one or more Z₁, heteroalkyleno, heteroalkyleno independently substituted with one or more Z₁, aryleno, aryleno independently substituted with one or more Z₁, heteroaryleno, and heteroaryleno independently substituted with one or more Z₁; and

Z₁ is selected from -R, halogen, -OS(O)₂OR, -SO₂OR, -SO₂R, -SO₂NR, -S(O)R, -OP(O)O₂RR, -P(O)O₂RR, -CO₂R, -NR₂, -NR₃, -NC(O)R, -C(O)R, -C(O)NR₂, -CN, -O and -OR, wherein R is independently selected from -H, alkyl, heteroalkyl, aryl, heteroaryl, arylalkyl, heteroarylalkyl and linking group.

47. The labelled nucleic acid compound of claim 46 wherein

Y_1 is taken together with R_1 or R_{11} and is C_2 or C_3 alkyleno or alkyleno independently substituted with one or more Z_1 ; or

Y_2 is taken together with R_1 or R_{11} and is C_2 or C_3 alkyleno or alkyleno independently substituted with one or more Z_1 ; or

Y_3 is taken together with R_4 or R_5 or R_6 or R_{13} and is C_2 or C_3 alkyleno or alkyleno independently substituted with one or more Z_1 ; or

Y_4 is taken together with R_4 or R_5 or R_6 or R_{13} and is C_2 or C_3 alkyleno or alkyleno independently substituted with one or more Z_1 .

48. The labelled nucleic acid compound of claim 47 wherein the C_2 or C_3 substituted alkyleno is gem disubstituted with C_1 - C_3 alkyl.

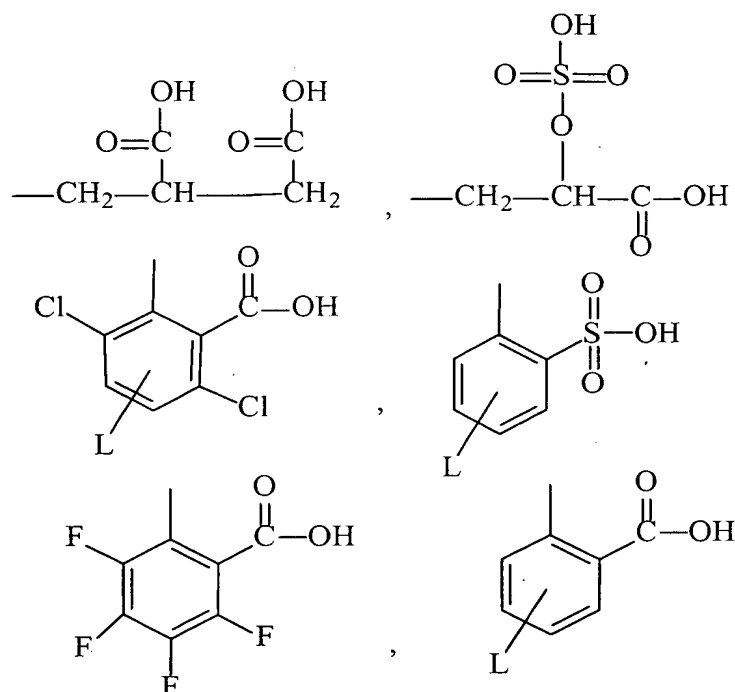
49. The labelled nucleic acid compound of claim 47 wherein the C_2 or C_3 substituted alkyleno is gem disubstituted with methyl.

50. The labelled nucleic acid compound of claim 46 wherein R_8 is alkyl independently substituted with one or more substituents selected from halogen, $-C(O)R$, and $-S(O)_2R$ wherein R is independently selected from $-OH$, O -alkyl, $-NH_2$, N -alkyl and a linkage.

51. The labelled nucleic acid compound of claim 46 wherein R_8 is $-CF_3$.

52. The labelled nucleic acid compound of claim 46 wherein R_8 is aryl or aryl independently substituted with one or more Z_1 .

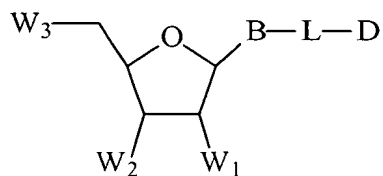
53. The labelled nucleic acid compound of claim 46 wherein R_8 is selected from the structures:



wherein L is a linkage.

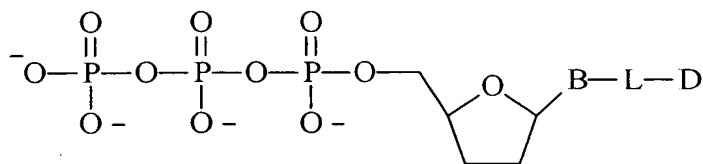
54. The labelled nucleic acid compound of claim 46 wherein NUC comprises a nucleobase selected from uracil, cytosine, deazaadenine, and deazaguanosine.

55. The labelled nucleic acid compound of claim 46 having the structure:



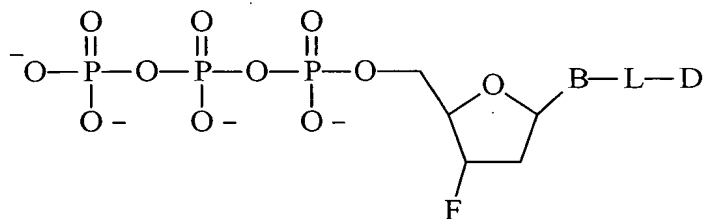
wherein B is a nucleobase; W_1 and W_2 taken separately are selected from $-H$, $-OH$, and $-F$; and W_3 is selected from $-OH$, monophosphate, diphosphate, triphosphate and phosphate analog

56. The labelled nucleic acid compound of claim 46 having the structure:



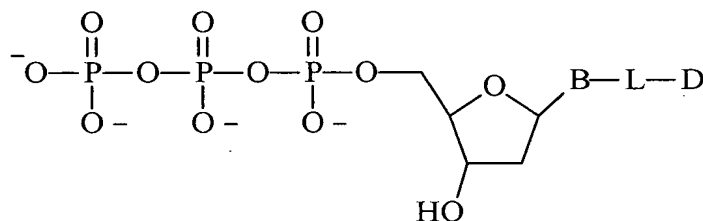
wherein B is a nucleobase.

57. The labelled nucleic acid compound of claim 46 having the structure:



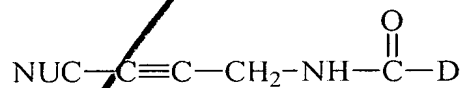
wherein B is a nucleobase.

58. The labelled nucleic acid compound of claim 46 having the structure:

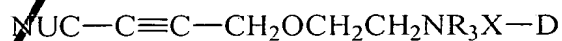


wherein B is a nucleobase.

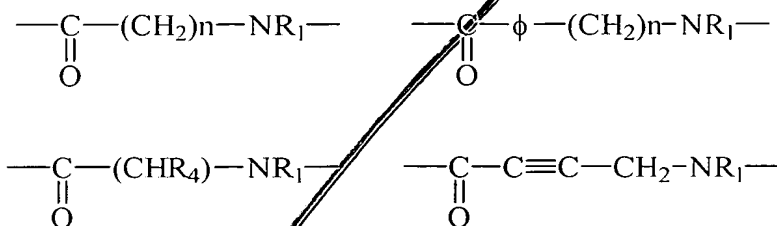
59. The labelled nucleic acid compound of claim 46 wherein L is attached to a nucleobase of NUC and to D in the structure:



60. The labelled nucleic acid compound of claim 46 wherein L is attached to a nucleobase of NUC and to D in the structure:



wherein R_3 is selected from $-H$ and (C_1-C_6) alkyl; and X is selected from the structures:



where n ranges from 1 to 5; ϕ is arylidyl; and R_1 is selected from $-H$, (C_1-C_6) alkyl and protecting group.

61. The labelled nucleic acid compound of claim 46 wherein L is attached at R_8 of D .

62. The labelled nucleic acid compound of claim 46 wherein NUC is a nucleotide and D is a donor dye and an acceptor dye wherein fluorescence energy transfer occurs between the donor dye and acceptor dye and at least one of the donor dye and acceptor dye is an extended rhodamine dye.

63. The labelled nucleic acid compound of claim 46 wherein NUC is a polynucleotide and L is attached to the polynucleotide at a position selected from the 5' terminus, the phosphodiester backbone, a nucleobase, and the 3' terminus.

64. The labelled nucleic acid compound of claim 63 wherein L is an aminohexyl linkage attached to the polynucleotide at the 5' terminus.

65. The labelled nucleic acid compound of claim 46 wherein NUC is a polynucleotide labelled with a donor dye and an acceptor dye wherein fluorescence energy transfer occurs between the donor dye and acceptor dye and at least one of the donor dye and acceptor dye is an extended rhodamine dye.

66. A method of PCR enzymatic synthesis comprising amplifying a template DNA with nucleotide triphosphates, polymerase, and two or more primers wherein the primers are complementary to the template DNA sequence and at least one of the primers is a labelled polynucleotide of claim 63.

67. A method of fragment analysis comprising the steps of:

forming one or more labeled polynucleotide fragments, the fragments being labeled with the labelled nucleic acid compound of claim 46;

resolving the one or more labeled polynucleotide fragments; and
detecting the resolved labeled polynucleotide fragments.

68. The method of claim 67 wherein the resolving step is an electrophoretic size-dependent separation process and the one or more labeled polynucleotide fragments are detected by fluorescence.

69. A kit for PCR enzymatic synthesis comprising one or more nucleotide triphosphates, polymerase, and two or more primers wherein one or more of the nucleotide triphosphates is a labelled nucleic acid compound according to claim 58.

70. A kit for PCR enzymatic synthesis comprising one or more nucleotide triphosphates, polymerase, and two or more primers wherein at least one of the primers is a labelled polynucleotide of claim 63.

71. A kit for fragment analysis comprising one or more nucleotide triphosphates, a chain-terminating nucleotide analog and a primer, wherein one or more of the nucleotide triphosphates is a labelled nucleic acid compound according to claim 55.

72. A kit for fragment analysis comprising one or more nucleotide triphosphates, a chain-terminating nucleotide analog and a primer, wherein one or more of the nucleotide triphosphates is a labelled nucleic acid compound according to claim 58.

73. A kit for fragment analysis comprising one or more nucleotide triphosphates, a chain-terminating nucleotide analog and a primer, wherein said chain-terminating nucleotide analog is a labelled nucleic acid compound according to claim 56.

74. A kit for fragment analysis comprising one or more nucleotide triphosphates, a chain-terminating nucleotide analog and a primer, wherein said chain-terminating nucleotide analog is a labelled nucleic acid compound according to claim 57.